I hereby certify that this correspondence is being filed via EFS-Web with the United States Patent and Trademark Office

on 18 Oct. 2010.

TOWNSEND and TOWNSEND and CREW LLP

By: Malinda Dagit

PATENT

Attorney Docket No.: 20695C-008700US

Client Ref. No.: 27900R1US BX2009T03678

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

I In re application of:

Váradi *et al.*

Application No.: 10/816,099

Filed: March 31, 2004

For: KITS FOR MEASURING THROMBIN GENERATION

Customer No.: 44183

Confirmation No. 9454

Examiner: Rosanne Kosson

Technology Center/Art Unit: 1652

APPELLANTS' BRIEF UNDER 37 CFR §41.37

Mail Stop Appeal Brief
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Further to the Notice of Appeal filed March 16, 2010, Appellants submit this Brief on Appeal. Also submitted with this brief is authorization to pay the fee as set forth in 37 C.F.R. §41.20(b)(2) and a petition with fee authorization for a five-month extension of time.

TABLE OF CONTENTS

1. REAL PARTY IN INTEREST

Baxter Healthcare S.A. and Baxter International Inc. are the assignees of the above-referenced patent application and therefore the real parties in interest.

2. RELATED APPEALS AND INTERFERENCES

None

3. STATUS OF CLAIMS

Claims 9, 14-21, and 24 are cancelled.

No claims are allowed.

No claims are objected to.

Claims 1-8, 10-13, 22, and 23 are rejected.

Claims 1-8, 10-13, 22, and 23 are being appealed.

4. STATUS OF AMENDMENTS

No claim amendments were made subsequent to the Final Office Action mailed December 16, 2009.

5. SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 1 relates to a kit for measuring the thrombin generation in a sample. The kit comprises (i) a lyophilized tissue factor (TF)/phospholipid (PL)-complex; and (ii) a lyophilized mixture comprising $CaCl_2$ and a thrombin substrate that has a Z-Gly-Gly-Arg-AMC fluorescent label. The lyophilized mixture (ii) is prepared from a solution comprising the substrate, $CaCl_2$, and DMSO. The lyophilized mixture has the property of forming a clear solution when dissolved in an amount of water that provides a concentration of 1 mM thrombin substrate and 15 mM $CaCl_2$. Support for independent claim 1 can be found, *e.g.*, at paragraphs **[028]**, **[048]**, and **[050]**.

Independent claim 22 relates to a method for measuring the thrombin generation in a whole blood or plasma sample where the method comprises providing a lyophilized TF/PL complex, and a lyophilized mixture that contains the thrombin substrate labelled with Z-Gly-Gly-Arg-AMC and $CaCl_2$. The lyophilized $CaCl_2$/substrate mixture is prepared from a solution comprising the thrombin substrate, $CaCl_2$ and DMSO. The lyophilized mixture has the property of forming a clear solution when dissolved in an amount of water that provides a concentration of 1 mM thrombin substrate and 15 mM $CaCl_2$. The lyophilized TF/PL complex and lyophilized mixture are contacted with a whole blood or plasma sample and thrombin generation is measured in the sample. Support for independent claim 22 can be found, *e.g.*, at paragraphs **[009]**, **[028]** and **[050]**; and at paragraphs **[048]**, **[014]**, and Figure 4.

6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The rejection of claims 1-8, 10-13, 22, and 23 as obvious over U.S. Patent No. 6,124,110 to Wöber *et al.* ("Wöber"s); in view of U.S. Patent No. 5,625,036 to Hawkins *et al.* ("Hawkins"); Váradi *et al., J. Thromb. Haemostasis* 1:2374-2380, 2003 ("Váradi"); U.S. Patent No. 5,952,198 to Chan ("Chan"); U.S. Patent No. 6,074,826 to Hogan *et al.* ("Hogan"); U.S. Patent No. 6,576,422 to Weinstein *et al.* ("Weinstein"); and U.S. Patent No. 6,756,019 to Dubrow *et al.* ("Dubrow") is being reviewed on appeal

7. ARGUMENT

A. The invention

The pending claims relate to a kit, and methods of using such a kit, that has two components: a lyophilized tissue factor (TF)/phospholipid (PL) complex and a lyophilized mixture that contains $CaCl_2$ and a fluorescent thrombin substrate together. Although the mixture is prepared from a solution that includes the fluorescent thrombin substrate, $CaCl_2$, and DMSO, the $CaCl_2$/fluorescent thrombin lyophilized mixture itself has the unexpected property of being dissolvable in water. In the current claims, the amount of water added results in a concentration of 1 mM thrombin substrate and 15 mM $CaCl_2$. The invention is based, in part, on the discovery

that it is not necessary to add an organic solvent such as DMSO when reconstituting the lyophilized $CaCl_2$/fluorescent thrombin mixture for use. This characteristic is not taught or suggested by the combination of references cited in the rejection, and moreover, as explained below, would not have been expected by a practitioner in this art.

B. The rejection

The rejection is summarized in the Final Office Action dated December 16, 2009. In brief, in the final rejection, the Examiner characterizes Wöber as disclosing TF as a dry powder and disclosing solutions of phosphatidylserine (PS) and phsphatidylcholine (PC). Wöber, along with Hawkins, are cited as allegedly disclosing various ratios of TF to PL. The Examiner further characterizes Wöber as disclosing the use of TF, PS, and PC to prepare a solution of vesicles containing TF and as indicating that the solution may be frozen. With regard to a lyophilized thrombin substrate and $CaCl_2$ preparation, Wöber is described in the rejection as disclosing a dry chromogenic thrombin substrate that is soluble in water, and that the thrombin reaction is initiated by the addition of $CaCl_2$ to the assay samples. The Examiner cites Váradi as teaching a fluorescent thrombin substrate.

The teachings of Chan, Hogan, Weinstein, and Dubrow are not characterized in the rejection set forth in the Final Office Action, although the Examiner points out specific passages of the references that allegedly support the rejection in the first Office Action dated August 1, 2005.

Chan relates to culturing mammalian cells in the presence of a liposome-like substance to increase recombinant Factor VIII production (*see*, abstract); Hogan relates to methods and kits for detecting *Borrelia* nucleic acids and indicates that enzymes, nucleotide triphosphates, primers and probes may be lyophilized (*see*, col. 37, lines 15-17); Weinstein relates to methods of detecting using reporter genes to detect a target of interest (col. 1, line 66 to col. 2, line 17); and Dubrow describes multifluidic devices which may contain a reagent immobilized onto a semi-permeable membrane using lyophilization (col 13, lines 5-6).

The Examiner contends that one of ordinary in the art at the time of the invention would have been motivated to lyophilize the TF/PL preparation of Wöber because Hawkins

teaches that this preparation is made as a reagent for performing prothrombin assays and is meant to be used for large scale clinical assays. The Examiner further alleges that one of skill would have substituted the fluorescent thrombin substrate of Váradi for the chromogenic substrate of Wöber because Wöber teaches that their substrate is available as a dry powder that is soluble in the buffer used in thrombin generation assay. The Examiner then asserts that lyophilized forms of these powders may also be prepared. The Examiner further alleges that one of ordinary skill in the art would have recognized the fluorescent substrate is interchangeable with the chromogenic sbustrate. The Examiner contends that one of skill would have been motivated to prepare a lyophilized reagent containing thrombin substrate and $CaCl_2$ based on Hawkins.

In the non-final Office Action mailed June 23, 2009, the Examiner had stated on page 4 that "the claimed kit and the method of its use, appear to be that used by Váradi et al. to measure thrombin generation time." Appellants note that there is no anticipation rejection of record and Appellants do not consider the invention to be disclosed in Váradi. However, during prosecution, in view of the Examiner's statement, Appellants provided a Declaration under 37 C.F.R. § 1.132 by all of the inventors stating that to the extent that the invention is disclosed in Váradi, it is their invention. In the Final Office Action dated December 16, 2009, the Examiner alleged that the Declaration by the inventors was insufficient to overcome the rejection and outlined various reasons that purportedly support this position. Although we disagree with the Examiner's legal reasoning, Appellants have not included arguments relating to this inventors' declaration in the present brief, as there is no formal rejection under 35 U.S.C. § 102 of record.

C. Legal standards

The Examiner has the burden of establishing a *prima facie* case of obviousness. The Supreme Court's ruling in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398, 82 USPQ 2d 1385 (2007) emphasized the principles set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966) regarding the framework for the objective analysis of obviousness under 35 U.S.C. § 103. The factual inquires enunciated in *Graham* for determining obviousness include determining the scope and contents of the prior art and ascertaining the differences between the

prior art and the claims in issue, and resolving the level of ordinary skill in the art. The rejection fails to properly determine the scope and content of the prior art in the context of the invention as a whole, and fails to properly ascertain the difference between the claimed invention and the prior art. Furthermore, in *KSR* the Supreme Court admonishes that obvious to try as a rationale to support a conclusion of obviousness is only applicable when choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success (When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense (*KSR Intl.*, 550 US 398, 402)). Here, the rejection fail to explain how each element of the invention as a whole is taught or suggested by the prior art and fails to provide a rationale as to why one of skill would have modified to the prior art to arrive at this invention. Moreover, there is no evidence presented in the rejection that, prior to Appellants' invention, one of skill would have expected that a lyophilized mixture comprising $CaCl_2$ and a fluorescent thrombin substrate having the characteristics recited in the claims would be soluble in an aqueous solution such as water or plasma. Thus, Appellants' invention is not one of a finite number of identified predictable solutions that would have reasonably been expected to work.

As detailed below, fluorescent thrombin substrates are not soluble in aqueous solutions. Given the insolubility of the substrates in aqueous solution and the precipitation of the substrate from aqueous solutions in the presence of $CaCl_2$, a practitioner in this art would have had no motivation to modify the cited references to lyophilize a suspension of the substrate with $CaCl_2$ with the intention of later reconstituting the dried material because there would have not been an expectation that a clear solution usable in an assay would result from the reconstitution. Appellants have demonstrated that a fluorescent thrombin substrate/$CaCl_2$ mixture can in fact be reconstituted in an aqueous medium without adding organic solvents. The invention as a whole is therefore unobvious over the cited art.

1. The rejection does not properly determine the scope and content of the prior art

The fluorescent substrates of the claimed invention are not soluble in aqueous solutions. (See, Appellants' response filed July 18, 2006, providing the Analytical Data Sheet for Z-Gly-Gly-Arg-AMC HCl as well additional scientific publications that show that the fluorescent thrombin substrates are insoluble in aqueous solution and must be first dissolved in methanol or a solution containing DMSO). Consistent with this insolubility in aqueous solutions, the instant specification explains that the addition of $CaCl_2$ to a fluorescent substrate in solution leads to formation of a precipitate (*see, e.g.*, paragraphs **[0028]** and **[0029]**). A Declaration under 37 C.F.R. § 1.132 by Peter Turecek (the "Turecek III Declaration) filed November 3, 2008, which is discussed in greater detail below, also explains the difficulties with solubility of the fluorescently-labeled thrombin. The Examiner omits this aspect of the invention from the analysis evaluating the scope and content of the prior art.

Further, the combination of references does not teach or suggest a lyophilized mixture comprising both $CaCl_2$ and a thrombin substrate comprising a fluorescent label. Wöber discloses methods of measuring thrombin generation using dried chromogenic substrates, but does not disclose or suggest use of a fluorescent substrate for measuring thrombin generation, much less a lyophilized mixture comprising $CaCl_2$ and the thrombin substrate. Hawkins describes a prothrombin reagent (PT) containing recombinant tissue factor, natural or synthetic phospholipids, calcium ion, and a buffer (*see*, Hawkins, col. 2, line 66 to col. 3, line 1). Hawkins indicates that tissue factor-containing reagents should be stable in a lyophilized state (*see*, col. 1, lines 43-50), but does not disclose or suggest anything regarding the nature of the substrates for a thrombin generation assay. Váradi discloses methods of measuring thrombin generation using fluorescent substrates, which are provided by the manufacturer as a dried powder and reconstituted in a DMSO-containing buffer (explained in Appellants' response filed January 31 2006), but does not disclose or suggest a lyophilized mixture that comprises $CaCl_2$ and a fluorescent thrombin substrate or using such a lyophilized mixture in the methods. All of the aforementioned references are completely devoid of any disclosure or suggestion of a lyophilized mixture comprising both $CaCl_2$ and a fluorescent thrombin substrate. There is no hint or suggestion in Wöber or Váradi that the thrombin substrates are lyophilized as a mixture with

$CaCl_2$. The substrates and $CaCl_2$ are distinct components of the reactions described in each of these references (*see, e.g.*, Wöber at col. 4, line 47 to col. 5, line 40; Váradi at page 2375, second full paragraph).

The Examiner notes that substrate preparations are available in dry powder and alleges that lyophilisation is an obvious alternative to dry powders. The Examiner also cites Wöber as teaching a chromogenic thrombin substrate that is soluble in water. However, the rejection fails to discuss the lack of solubility of the fluorescent thrombin substrates in water. The fact that a chromogenic thrombin substrate may be soluble in water is irrelevant to the claimed invention. Although the Examiner proposes lyophilisation of a fluorescent thrombin substrate as a simple alternative to preparing a dried powder, there is no discussion of the differences in solubility. As explained in the instant specification, addition of $CaCl_2$ to a fluorescent substrate leads to formation of a precipitate (*see, e.g.*, page 9, paragraph 28 to page 10, paragraph 29). Thus, one of skill in the art would not expect that a lyophilized mixture of $CaCl_2$ and a fluorescent substrate prepared as described in the specification could be dissolved in an aqueous solution to form a clear solution. Thus, the rejection also fails to consider this aspect of the invention when addressing the scope and content of the prior art.

In addition, the disclosures of Chan, Hogan, Weinstein, and Dubrow are not relevant to detection of any protein activity, much less thrombin activity. The methods of Weinstein may employ a lyophilized "cell or viral detector composition" (*i.e.*, reporter gene) immobilized on a solid support (*see*, col. 16, lines 4-11), but do not suggest a lyophilized mixture comprising $CaCl_2$ and a fluorescent thrombin substrate. Dubrow describes multifluidic devices which may contain a reagent immobilized onto a semi-permeable membrane using lyophilization (col 13, lines 5-6), but does not disclose a lyophilized mixture comprising $CaCl_2$ and a fluorescent thrombin substrate. Neither Chan nor Hogan provide any teachings relating to a lyophilized mixture.

Further, given the insolubility of the recited fluorescent substrate in the absence of an organic solvent, the rejection fails to provide a clear articulation of why one of skill would have made the proposed modification to substitute an insoluble, fluorescent thrombin substrate for a soluble chromogenic thrombin substrate, prepare a mixture with $CaCl_2$ and then lyophilize

it with the expectation of obtaining a mixture that is dissolvable in water. No evidence or reasoning is provided in the rejection that one of skill would consider this to be a reasonable modification of the cited art.

In view of the foregoing, the rejection fails to properly establish the scope and content of the prior art and fails to provide a clear articulation of the reasons for modifying the cited art in a way that would arrive at the invention claimed here.

2. No reasonable expectation of success

Even assuming *arguendo* that one of skill would have been tempted to combine the references as suggested by the Examiner, the skilled artisan would have no reasonable expectation of success in arriving at the claimed invention. As discussed above and further elaborated on below with regard to the Turecek III Declaration, the addition of $CaCl_2$ to a fluorescent substrate in aqueous solution leads to formation of a precipitate. Accordingly, the skilled artisan would expect that lyophilization of a fluorescent substrate in the presence of $CaCl_2$ would result in a precipitate when such a lyophilized powder is reconstituted with an aqueous buffer or water, not the clear solution as presently claimed.

As explained in detail in the instant specification and by Dr. Turecek in the Turecek III Declaration, a lyophilized mixture as recited in the claims has the property of being readily soluble in an aqueous solution. The evidence provide by Dr. Turecek further indicates that the DMSO concentration of the solution prior to lyohilization is not determinative of the increased solubility of such a mixture in water (or plasma or serum). As one of skill in this art, Dr. Turecek additionally attests that the ready solubility of the lyophilized fluorescent substrate/$CaCl_2$ in an aqueous solution was surprising. Without the teachings of the instant specification, the skilled artisan would expect that lyophilization of a fluorescent substrate in the presence of $CaCl_2$ would result in a precipitate when such a lyophilized preparation is reconstituted with an aqueous buffer or water, not the clear solution. Thus, one of skill would not have expected to be able to obtain a lyophilized mixture having the characteristics recited in the claims.

The Turecek III Declaration

The Turecek III Declaration is the third Declaration presented regarding the issue of solubility of the $CaCl_2$/fluorescent thrombin substrate mixture. We believe that this Declaration is sufficient to address the rejection of the current claims and therefore have not included the two preceding Turecek Declarations with this Brief.

First, it is noted that the experimental data provided in the Turecek III Declaration evaluates the solubility in the same vial type (glass) so that each of the sample evaluation and re-solubilization procedures is performed in the same type of container. Appellants further note that Example 4, paragraph 48 of the specification provides further evidence that a lyophilized mixture comprising the fluorescent thrombin substrate and $CaCl_2$ that is lyophilized in a microtiter plate (as opposed to glass vials used for the Turecek III Declaration experiments) is also soluble in an aqueous solution to which DMSO has not been added. The data in Example 4 in the specification show thrombin generation where the lyophilized TF/PL-complex and the lyophilized thrombin substrate/$CaCl_2$ mixture are present together in one well of a microtiter plate in comparison to where the lyophilized TF/PL-complex is lyophilized separately from the lyophilized thrombin substrate/$CaCl_2$ mixture. Regardless of whether the thrombin substrate/$CaCl_2$ is lyophilized with the TF/PL-complex or separately from the TF/PL complex, it is soluble in an aqueous solution, *e.g.,* plasma, without DMSO (see, Figure 4 of the applciation).

It is noted that the original photographs in Exhibit 2 of the Turecek III Declaration are in color. The differences in the solutions are clearly visible in the black and white version of the photographs in our files that were filed with this response. The Examiner had indicated, however, that the photographs were difficult to see in the black and white version of the photographs that is available in the Image File Wrapper at the USPTO. Appellants therefore supplied color copies of the photographs as arranged with the Examiner in an e-mail to the Examiner submitted on June 1, 2009. Appellants believe that these photographs are also accessible to other USPTO personnel, however, are happy to arrange to provide further color copies if requested to do so.

As explained in the Turecek III Declaration, different samples were prepared as indicated on the flow sheet provided as Exhibit 1 of the Declaration. For all of the experiments,

the solutions were prepared and analyzed in glass sample containers to directly compare the solubility of the samples. Solubility was assessed visually (see, *e.g.,* the photographs in Exhibit 2 of the Turecek III Declaration.

a. Experimental protocol, Turecek III Declaration

Initially, 250 mg of the fluorescent-labeled thrombin substrate Z-Gly-Gly-Arg-AMC.HCL powder was weighed in a 100 ml flask and dissolved by stirring with a magnetic stirrer in 74 ml of 25 mM HEPES, 175 mM NaCl pH 7.35 buffer, containing 10% DMSO. After the powder was fully dissolved, 6 ml water was added to bring the volume of the substrate solution to 80 ml. The solution remained clear upon the addition of the water. This solution has a substrate concentration of 5 mM in 24 mM HEPES, 175 mM NaCl – 9.25% DMSO buffer. The solution was further processed as shown in the flow chart in Exhibit 1 attached to the Declaration and as summarized below.

The 80-ml substrate solution containing substrate was divided into parts and aliquoted as shown in the flow chart. This procedure resulted in four different substrate samples, two of them lyophilized without $CaCl_2$ and two of them lyophilized after the addition of $CaCl_2$ to the substrate solution. These samples are as follows.

Sample 1: The concentration of substrate in sample 1 (prior to lyophilization) was 5 mM with 10% DMSO--no $CaCl_2$ is present in the lyophilized sample;

Sample 2: The concentration of substrate in sample 2 (prior to lyohpilization) was 2.5 mM, 5% DMSO--no $CaCl_2$ is present in the lyophilized sample;

Sample 3: The concentration of substrate in sample 3 (prior to lyophilization) was 1 mM. The solution that was lyophilized also contained 15 mM $CaCl_2$ and 2% DMSO; and

Sample 4: The concentration of substrate, $CaCl_2$, and DMSO (prior to lyophilization) is the same as Sample 3; the concentration of the buffer components are different.

Sample 4 had the same composition as described in Example 6 (starting at paragraph 50) in the instant specification. During the preparation of the calcium-containing samples no precipitation was visible upon the addition of $CaCl_2$ dissolved either in water

(Sample 3) or in buffer (Sample 4) prior to lyophilization. The solutions containing the thrombin substrate without $CaCl_2$ for samples 1 and 2 were also clear prior to lyophilization.

The vials were reconstituted and prepared to reflect a "ready to use" solution, containing 1 mM fluorescent substrate and 15 mM $CaCl_2$ in different experiments. Each resolubilization procedure was performed at least in duplicate.

b. Experimental results, Turecek III Declaration

In the experimental series described in the Declaration, the substrate was dissolved and diluted before the addition of $CaCl_2$. In one set of samples (samples 1 and 2), the substrate (clear solution) was lyophilized before the addition of $CaCl_2$, such that the solubility of lyophilized substrate without the $CaCl_2$ could be compared to the solubility of the lyophilized substrate mixture that contains $CaCl_2$. When the substrate was lyophilized without $CaCl_2$ present in the mixture, the lyophilized powder was largely insoluble (it required long and vigorous shaking, mixing and warming to dissolve) (see, reconstitution experiments 1, 1a, and 2). As a consequence, this kind of product would not be regarded as a "ready to use " reagent.

In the other experiments (samples 3 and 4), the $CaCl_2$ was added to the diluted, dissolved substrate (no precipitate was visible) and then the substrate and $CaCl_2$ were lyophilized together. These lyophilized mixture could easily be reconstituted with water for injection (WFI).

Experiments 1a, Experiment 3, and Experiment 4 are of particular interest in analyzing solubility employing an amount of water to reconstitute the lyophilized preparation that results in the concentrations of $CaCl_2$ and fluorescent thrombin substrate recited in the current claims.

Summary of comparative results Experiments 1a, 3, and 4

Sections 8-12 of the Turecek III Declaration described the results of the experiments. Table 1, which accompanied Appellants' response filed February 19, 2009, and is presented again in this Brief, provides a summary of Experiments 1a, 3, and 4 presented in the Turecek Declaration in tabular form for ease of comparison. The volume of solution used in resuspending the lyophilized mixture for each of experiments 1a, 3, and 4 was selected to

provide a final concentration of 1 mM fluorescent thrombin substrate and 15 mM $CaCl_2$, as set forth in the claims.

Table 1: Comparison of Experiments Described in the Turecek Declaration

Experimental Step	**Experiment 1a (Sample 1)**	**Experiment 3 (Sample 3a)**	**Experiment 3 (Sample 3b)**	**Experiment 4 (Sample 4)**
Starting Solution Concentrations	5 mM fl-thrombin 10% DMSO	1 mM fl-thrombin 2% DMSO 15 mM **$CaCl_2$**	1 mM fl-thrombin 2% DMSO 15 mM **$CaCl_2$**	1 mM fl-thrombin 2% DMSO 15 mM **$CaCl_2$** 25 mM HEPES 170 mM NaCl
Lyophilization Step	Yes	Yes	Yes	Yes
Resuspension Volume & Solution Type	5 mL 15 mM **$CaCl_2$** *$CaCl_2$ added post-lyophilization.*	1 mL water	0.2 mL of water + 0.8 mL of 25 mM HEPES/170 mM NaCl	1 mL of water
Final Solution Concentrations*	1 mM fl-thrombin 2% DMSO 15 mM $CaCl_2$	1 mM fl-thrombin 2% DMSO 15 mM $CaCl_2$	1 mM fl-thrombin 2% DMSO 15 mM $CaCl_2$ 20 mM HEPES 136 mM NaCl	1 mM fl-thrombin 2% DMSO 15 mM $CaCl_2$ 25 mM HEPES 170 mM NaCl
Clear or Cloudy Final Solution	***Cloudy;*** *Clear **only*** after vigorous mixing and heating to 37°C	***Clear***; easily dissolved after short vortexing	***Clear*** upon dilution with buffer	***Clear***; dissolved immediately in water

Table 1 Key: fl-thrombin = fluorescently labeled thrombin; Sample and Experiment numbers correspond to those described in the Turecek Declaration. *Final concentrations of DMSO are approximate.

The sample employed in Experiment 1a (Sample 1) had the following concentrations prior to lyophilization: 5 mM fluorescent thrombin substrate and 10% DMSO. The substrate was dissolved and the clear solution was then lyophilized—no $CaCl_2$ was present in the [clear] solution prior to lyophilization and hence, the lyophilized aliquots of this sample. In experiment 1a, an aliquot of this lyophilized mixture was resuspended in 5 mls of 15 mM $CaCl_2$ (to provide a solution where the final volume of substrate is 1 mM and the $CaCl_2$ concentration is 15 mM). However, the substrate was barely soluble (see, section 9 of the Turecek Declaration, as well as Table 1, column 2).

One can directly contrast Experiment 1a with Experiments 3 and 4. For the purposes of comparing concentrations of the fluorogenic thrombin substrate and $CaCl_2$, the discussion focuses on Experiment 3, but the same observations hold true for Experiment 4. In Sample 3, the concentration of fluorogenic thrombin substrate prior to lyophilization was 1 mM. The solution also contained 15 mM $CaCl_2$ and 2% DMSO. The thrombin substrate was dissolved and the clear solution was lyophilized. For Experiment 3a, an aliquot was resuspended in a volume of 1 ml of water (section 11 of the Turecek Declaration and Table 1, column 3). This 1 ml provides a final concentration of 1 mM substrate and 15 mM $CaCl_2$, as set forth in the claims. The mixture was easily dissolved (Turecek Declaration, section 11a, Experiment 3a; Table 1, column 3). Even if re-suspended in 0.2 ml of water followed by adding HEPES buffer (Turecek Declaration, section 11b, Experiment 3b; Table 1, column 4), the initially opalescent mixture was readily dissolved when the volume was brought to 1 ml with HEPES buffer.

This same "readily soluble" property of the lyophilized substrate/$CaCl_2$ mixture was also shown in Experiment 4 (reconstituted with 1 ml of water) where the lyophilized mixture was prepared using a more concentrated HEPES buffer with a higher NaCl concentration (section 12; Table 1, column 5).

Dr. Turecek then explains that the ready solubility of the lyophilized fluorescent substrate/$CaCl_2$ mixture was a surprising finding based on his experience working with fluorescent-labeled thrombin substrates.

In the Office Action mailed November 21, 2008, the Examiner states that the results are not truly surprising because DMSO may remain following lyophilization and that is why the claimed mixture is soluble. Appellants do not necessarily agree with this analysis, nor do Appellants concede that this is relevant to the surprising solubility of the lyophilized mixture. However, it is noted that while Sample 1 contains the highest starting concentration of DMSO (10%) it is the least soluble (section 9 compared to section 11; Table 1, column 2 compared to columns 3-4), indicating that the DMSO concentration of the solution prior to lyophilization is not determinative of the increased solubility observed for sample 1a in comparison to samples 3 and 4.

3. The evidence supports a legal conclusion of unobviousness

"In determining the differences between the prior art and the claims, the question under 35 U.S.C. § 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious." (*Stratoflex, Inc. v. Aeroquip Corp.,* 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983); *Schenk v. Nortron Corp.*, 713 F.2d 782, 218 USPQ 698 (Fed. Cir. 1983). The rejection only establishes that various isolated individual elements (fluorogenic thrombin substrates, the existence of a technique to assay thrombin activity, the existence of lyophilization as a process for preparing various compositions, etc.) were known in the art, but fails to provide a clear articulation of why these disparate disclosures would lead one of skill to Appellants' invention.

Although any differences between the claimed invention and the prior art may be expected to result in some difference in properties, the issue is whether the properties differ to such an extent that the difference is really unexpected. Here, fluorescent thrombin substrates are known to have only limited solubility in water. One of skill would logically have expected that this would hold true when the substrate is lyophilized together as a mixture with the $CaCl_2$. The inventors discovered that this was not the case. In addition to the deficiencies in the rejection explained above, this unexpected result is sufficient in itself to establish unobviousness (absence of an expected property is evidence of nonobviousness, *Ex parte Mead Johnson & Co.* 227 USPQ 78 (Bd. Pat. App. & Inter. 1985).

As discussed above and as Dr. Turecek explains in the Turecek Declaration, the ready solubility of the lyophilized fluorescent substrate/$CaCl_2$ mixture in Experiments 3 and 4 was surprising. Appellants have provided comparative evidence that supports that this is an unexpected property of the claimed lyophilized mixture.

4. Claims 22 and 23 are separately patentable

Claims 22 and 23 recite contacting a whole blood or plasma sample with the lyophilized TF/PL-complex and the lyophilized mixture containing thrombin-substrate and $CaCl_2$ and measuring the thrombin generation in the sample. Claims 22 and 23 are patentable for the reasons explained above. In addition, the claims are separately patentable because the

rejection does not establish evidence or reasoning that one of skill could actually successfully perform the claimed method. As explained above, one of skill would not have expected the lyophilized $CaCl_2$/thrombin-substrate mixture to be soluble when an aqueous solution that does not contain an organic solvent is added. Therefore, one of skill would not have expected to be able to successfully measure the thrombin generation in the whole blood or plasma sample that is used to dissolve the lyophilized preparation. Claims 22 and 23 are therefore additionally patentable over the art cited in the rejection.

8. CONCLUSION

For the reasons explained above, the rejection only establishes that some of the isolated individual elements recited in the claims were known in the art, but fails to provide a clear articulation of why the disparate disclosures describing these elements would lead to Appellants' invention. Moreover, the rejection fails to provide any explanation as to why one of skill would have expected the modifications proposed by the Examiner to result in a lyophilized mixture comprising $CaCl_2$ and fluorescent thrombin substrate with the solubility characteristics set forth in the claims. Accordingly, under the standard of obviousness articulated by the Supreme Court in *KSR International Co. V. Telefex, supra,*, the claims are patentable.

In view of the foregoing, it is therefore respectfully submitted that the rejection should be reversed.

Respectfully submitted,

Jean M. Lockyer, Ph.D.
Reg. No. 44,879

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300

62819973 v1

9. CLAIMS APPENDIX

1. (previously presented) A kit for measuring the thrombin generation in a sample, said kit comprising

(i) a lyophilized tissue factor (TF)/phospholipid (PL)-complex; and

(ii) a lyophilized mixture comprising $CaCl_2$ and a thrombin substrate that comprises a fluorescent label, where said thrombin substrate that comprises the fluorescent label is Z-Gly-Gly-Arg-AMC; wherein the lyophilized mixture is prepared from a solution comprising the substrate, $CaCl_2$ and DMSO and forms a clear solution when dissolved in water, and further, wherein the amount of water that dissolves the lyophilized mixture to form the clear solution provides a concentration of 1 mM thrombin substrate and 15 mM $CaCl_2$.

2. (original) The kit according to claim 1, wherein the concentration of TF in the lyophilized TF/PL-complex ranges from about 5 to about 1000 pM.

3. (original) The kit according to claim 1, wherein the concentration of PL in the lyophilized TF/PL-complex ranges from about 1 to about 100 μM.

4. (original) The kit according to claim 1, wherein said TF or at least a functional part thereof is of natural or recombinant origin.

5. (original) The kit according to claim 1, wherein said PL is of natural or synthetic origin.

6. (original) The kit according to claim 1, wherein said PL is selected from the group consisting of phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and mixtures thereof.

7. (original) The kit according to claim 6, wherein the weight ratio of PC/PS is in the range of from about 60/40 to about 95/5.

8. (original) The kit according to claim 6, wherein the weight ratio of PC/PS/PE is in the range of from about 60/20/20 to about 78/17/5, based on the total amount of phospholipids.

9. (cancelled)

10. (original) The kit according to claim 1, further comprising at least one thrombin standard.

11. (original) The kit according to claim 1, wherein the lyophilized TF/PL-complex is immobilized onto a support.

12. (previously presented) The kit according to claim 1, wherein the lyophilized mixture comprising $CaCl_2$ and the thrombin-substrate comprising a fluorescent label is immobilized onto a support.

13. (original) The kit according to claim 11 or 12, wherein the support is the inner surface of a vial or wells of an ELISA plate or strip.

14-21. (cancelled)

22. (previously presented) A method for measuring the thrombin generation in a whole blood or plasma sample, comprising the steps of:

(a) providing a lyophilized tissue factor (TF)/phospholipid (PL)-complex and a lyophilized mixture containing a thrombin-substrate that comprises a fluorescent label, where said thrombin substrate that comprises the fluorescent label is Z-Gly-Gly-Arg-AMC, and $CaCl_2$, wherein the lyophilized mixture is prepared from a solution comprising the thrombin substrate, $CaCl_2$ and DMSO and forms a clear solution when dissolved in water, wherein the amount of water that dissolves the lyophilized mixture to form the clear solution provides a concentration of 1 mM thrombin substrate and 15 mM $CaCl_2$;

(b) contacting the whole blood or plasma sample with said lyophilized TF/PL-complex and said lyophilized mixture containing thrombin-substrate and $CaCl_2$; and

(c) measuring the thrombin generation in said sample.

23. (previously presented) The method according to claim 22, wherein the sample is a cell-free plasma sample.

24. (cancelled)

10. EVIDENCE APPENDIX

A. Declaration under 37 C.F.R. § 1.132 by Dr. Peter Turecek

a) An unsigned version of the Declaration with exhibits was submitted with Appellants' response filed November 6, 2008 to the Office Action mailed May 1, 2008. A signed version of the Declaration was filed on November 11, 2008. The signed version of the Declaration with the exhibits initially filed with the unsigned version is provided in this appendix.

b) the Final Office Action mailed November 21, 2008 acknowledged receipt of both versions of the filed Declaration.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Varadi *et al.* Application No.: 10/816,099 Filed: March 31, 2004 For: KITS FOR MEASURING THROMBIN GENERATION Customer No.: 44183	Confirmation No. 9454 Examiner: Rosanne Kosson Technology Center/Art Unit: 1653 DECLARATION UNDER 37 C.F.R. § 1.132 BY DR. PETER TURECEK

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Peter Turecek, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

2. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

3. I am a named inventor on the above-referenced patent application. I have previously submitted two Declarations under 37 C.F.R. § 1.132 in support of the patentability of this application over various references cited in an obviousness rejection. However, the Examiner contends that the previous Declarations are insufficient to overcome the rejection (see, the Office Action dated May 1, 2008). My understanding of the reasons is as follows: First, the Examiner alleges that a proper comparison was not provided in the previous Declarations

because no data were presented that compared the solubility of a lyophilized mixture that contains both the substrate and $CaCl_2$ with the solubility of a lyophilized substrate where the substrate is lyophilized separately without the $CaCl_2$. Next, the Examiner also alleges that the previous Declarations were insufficient because the data were obtained using higher concentrations of fluorescent thrombin substrate and $CaCl_2$ solutions than the final concentration in the cited reference, Váradi *et al.* Last, the Examiner also contends that the data previously submitted suggested that the solubility in those experiments was also influenced by the container, *i.e.,* whether or not the substrate/$CaCl_2$ is prepared in glass vials or plastic wells. This Declaration is provided to further address these issues.

Preparation of Substrate Solutions

4. In the experiments presented here, different substrate samples were prepared as indicated on the attached flow sheet (Exhibit 1) to provide four samples that were directly compared in terms of solubility. For all of the experiments, the solutions were prepared and analyzed in glass sample containers to directly compare the solubility of the samples. Solubility was assessed visually (see, the photographs provided in Exhibit 2 for the various re-solubilization experiments described below).

5. Initially, 250 mg of the fluorescent-labeled thrombin substrate Z-Gly-Gly-Arg-AMC.HCL powder was weighed in a 100 ml flask and dissolved by stirring with a magnetic stirrer in 74 ml of 25 mM HEPES, 175 mM NaCl pH 7.35 buffer, containing 10% DMSO. After the powder was fully dissolved, 6 ml water was added to bring the volume of the substrate solution to 80 ml (in the experiments in the previous declarations, 6 ml of 1M $CaCl_2$ was added instead of the water). The solution remained clear upon the addition of the water. This solution has a substrate concentration of 5 mM in 24 mM HEPES, 175 mM NaCl – 9.25% DMSO buffer. The solution was further processed as shown in the flow chart in Exhibit 1.

6. The 80-ml substrate solution containing substrate was divided into parts and aliquoted as shown in the flow chart. This procedure resulted in four different substrate samples, two of them lyophilized without and two of them lyophilized after the addition of $CaCl_2$. The concentrations of substrates in the four samples are as follows:

Sample 1: The concentration of substrate in sample 1 (prior to lyophilization) was 5 mM with 10% DMSO--no $CaCl_2$ is present in the lyophilized sample;

Sample 2: The concentration of substrate in sample 2 (prior to lyohpilization) was 2.5 mM, 5% DMSO--no $CaCl_2$ is present in the lyophilized sample;

Sample 3: The concentration of substrate in sample 3 (prior to lyophilization) was 1 mM. The lyophilized sample also contains 15 mM $CaCl_2$ and 2% DMSO; and

Sample 4: The concentrations of substrate, $CaCl_2$, and DMSO are the same as Sample 3; the concentrations of the buffer components are different.

Sample 4 had the same composition as described in Example 6 (starting at paragraph 50) in the patent application. This is the minimum concentration of the substrate and $CaCl_2$ used here. During the preparation of the calcium-containing samples no precipitation was visible upon the addition of $CaCl_2$ dissolved either in water (Sample 3) or in buffer (Sample 4) prior to lyophilization. The solutions containing the thrombin substrate without $CaCl_2$ for samples 1 and 2 were also clear prior to lyophilization.

Reconstitution of the Lyophilized substrate Samples

7. The vials were reconstituted and prepared to reflect a "ready to use" solution, containing 1 mM fluorescent substrate and 15 mM $CaCl_2$ in different experiments, as described below. Each resolubilization procedure was performed at least in duplicate.

8. *Experiment 1*

The lyophilized powder in the vials of Sample 1 (5 mM Substrate prepared in 25 mM HEPES, 175 mM NaCl + 10% DMSO – Buffer) strongly adhered to the wall of the vials. After addition of 1 ml water for injection (WFI) the substrate was barely soluble, the powder continued to stick on the glass wall of the vial. To obtain a clear solution we had to vigorously mix the solution with a vortex and warm it to 37°C.

After the substrate was dissolved in the 1 ml of water, the required amount of $CaCl_2$ to reach a final concentration of 15 mM $CaCl_2$ was added in three different ways:

a. 4 ml of 19 mM $CaCl_2$ solution (in water) was added;

b. 4 ml of 19 mM $CaCl_2$ in 25 mM HEPES, 175 mM NaCl pH 7.35 buffer was added; or

c. First 1 ml of 25 mM HEPES, 175 mM NaCl pH 7.35 buffer was added, followed by 3 ml of 25 mM $CaCl_2$.

No opalescence or precipitation was observed upon the addition of $CaCl_2$ to the dissolved substrate.

9. *Experiment 1a*

As noted above, the lyophilized powder in vials of Sample 1 (5 mM Substrate prepared in 25 mM HEPES, 175 mM NaCl + 10% DMSO – Buffer) strongly adhered to the wall of the vials and was present on the glass wall. In order to compare the solubilizing protocol in Experiment 1 to a protocol using one step to bring the solution to the desired concentrations, 5 mls of 15 mM $CaCl_2$ were added to the lyophilized powder to attempt to re-solubilize it. As in Experiment 1 using 1 ml of water, the substrate was barely soluble, the powder was sticking to the glass wall of the vial. To obtain a clear solution, we again had to vigorously mix the solution using a vortex and warm it to 37°C.

10. *Experiment 2*

The lyophilized powder in the vials of Sample 2 (2.5 mM Substrate prepared in 25 mM HEPES, 175 mM NaCl -5 % DMSO buffer) also strongly adhered to the wall of the vials. After addition of 1 ml water for injection (WFI), the substrate was barely soluble, the powder was sticking to the glass wall of the vial. To obtain a clear solution, it was also necessary to vortex it vigorously and warm the solution to 37°C.

After the substrate was dissolved, the amount of $CaCl_2$ to bring the final concentration to 15 mM was added in two buffer media:

a. 1.5 ml of 25 mM $CaCl_2$ solution (in water) was added; or

b. 1.5 ml of 25 mM $CaCl_2$ solution 25 mM HEPES, 175mM NaCl pH 7.35 buffer was added.

No opalescence or precipitation was observed upon the addition of $CaCl_2$ to the dissolved substrate in either case.

11. *Experiment 3*

The lyophilized powder in the vials of Sample 3 (1 mM Substrate; 15 mM $CaCl_2$ in 10 mM HEPES; 70 mM NaCl; -2% DMSO buffer) formed a thin film that was visible at ~ 2/3 of the glass wall. Because this sample had a lower buffer concentration, two different dissolving strategies were applied:

a. Reconstitution with 1 ml of water for injection (WFI). In contrast to the Experiments 1 and 2, after addition of 1 ml of water the substrate easily dissolved after a short vortexing.

b. Reconstitution with 0.2 ml of water for injection (WFI), followed by the addition of 0.8 ml of 25 mM HEPES, 170mM NaCl pH 7.35 buffer. The addition of the small volume of WFI resulted in an opalescent solution, which readily became clear upon dilution to the original lyophilization volume (1 ml) with the buffer.

12. *Experiment 4*

The composition of the lyophilized powder in the vials of Sample 4 (1 mM Substrate; 15 mM $CaCl_2$ in 25 mM HEPES; 175 mM NaCl; -2% DMSO buffer) was similar to those described in Example 6 of the specification.

Upon reconstitution with 1 ml of water for injection (WFI), the lyophilized powder dissolved immediately, resulting in a "ready to use" reagent for TGA.

Conclusion

13. The present invention relates to the discovery of a procedure where a non-water-soluble fluorescence substrate can be converted to a water-soluble, "ready to use" reagent. In various examples, including Example 6 of the specification, the procedure includes a step in which $CaCl_2$ is added to a concentrated substrate solution, resulting in a precipitate formation, which can only be dissolved with difficulties. After re-solubilization and dilution to the desired concentrations, the substrate-$CaCl_2$ solution is lyophilized as a mixture, and, surprisingly, the resulting powder becomes very easy to dissolve in water.

14. In the experimental series presented in this Declaration, the substrate was dissolved and diluted (see, initial preparation procedure) before the addition of $CaCl_2$. In one set of samples (samples 1 and 2), the substrate (clear solution) was lyophilized before the addition of $CaCl_2$, such that the solubility of lyophilized substrate without the $CaCl_2$ could be compared to the solubility of the lyophilized substrate mixture that contains $CaCl_2$. When the substrate was lyophilized without $CaCl_2$ present in the mixture, the lyophilized powder was largely insoluble (it required long and vigorous shaking, mixing and warming to dissolve) (see, reconstitution experiments 1, 1a, and 2). As a consequence, this kind of product would not be regarded as a "ready to use " reagent.

15. In the other experiments (samples 3 and 4) in which the $CaCl_2$ was added to the diluted, dissolved substrate (no precipitate was visible) and then the substrate and $CaCl_2$ were lyophilized together, the lyophilized mixture could easily be reconstituted with water for injection (WFI).

16. The experiments presented in this Declaration thus provide additional evidence that it is necessary to lyophilize the substrate together with the $CaCl_2$ to produce an easy water-soluble "ready to use" substrate reagent. Based on my experience in this field working with fluorescent-labeled thrombin substrates, this is a new, surprising effect to convert a non-water-soluble product to a water-soluble product.

17. The Declarant has nothing further to say.

Date: Nov. 7. 2008

By: _____
Peter Turecek, Ph.D.

61655589 v1

EXHIBIT 1
DECLARATION UNDER 37 C.F.R. § 1.132 BY DR. PETER TURECEK

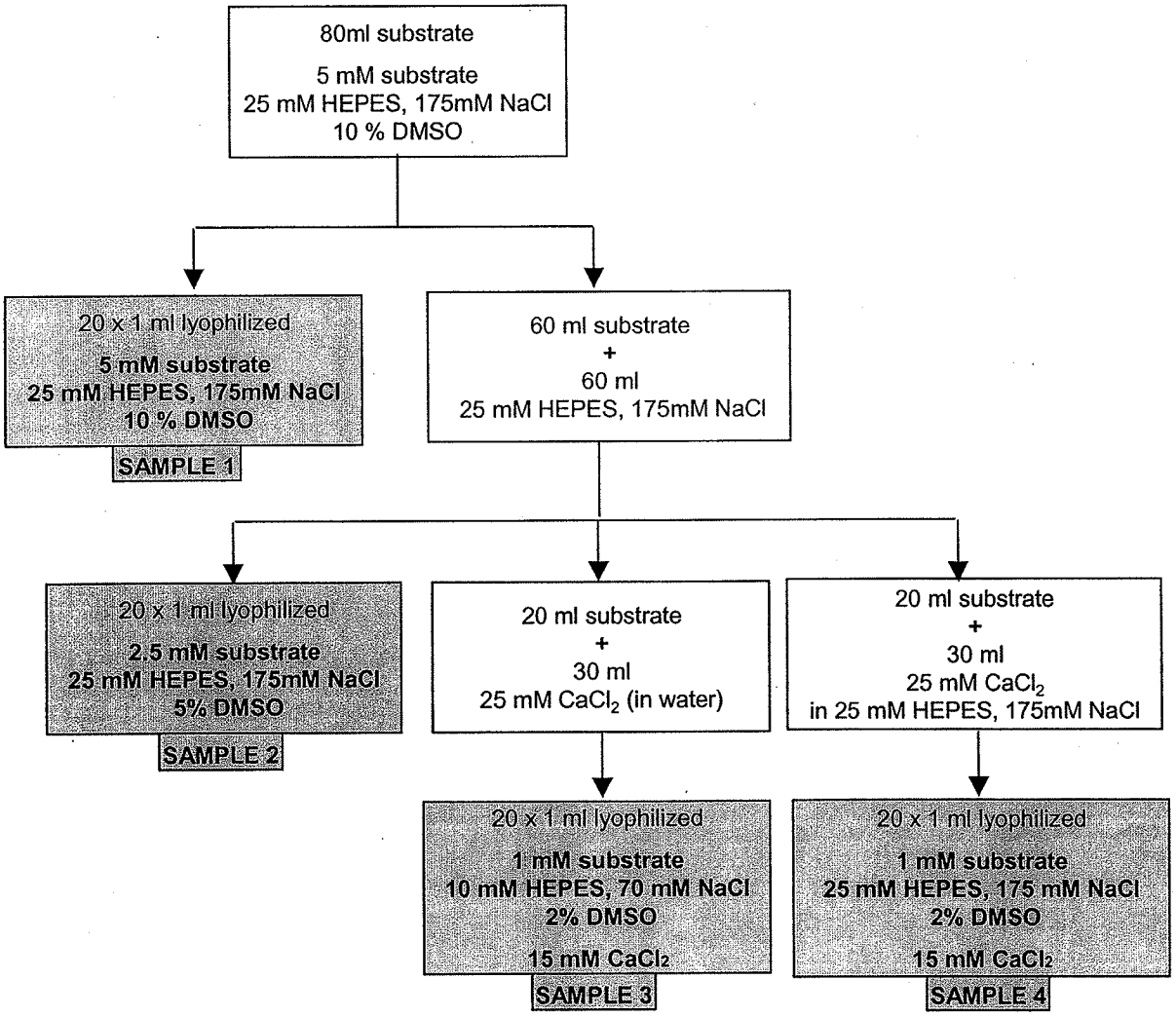
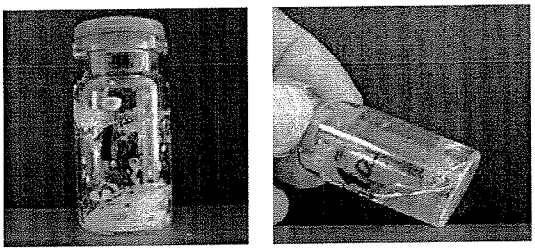


EXHIBIT 2
DECLARATION UNDER 37 C.F.R. § 1.132 BY DR. PETER TURECEK

Pictures to Experiment 1



Picture to Experiment 1a

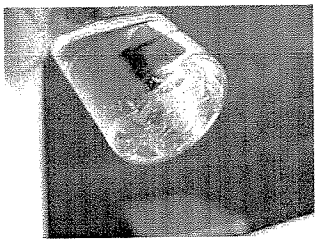
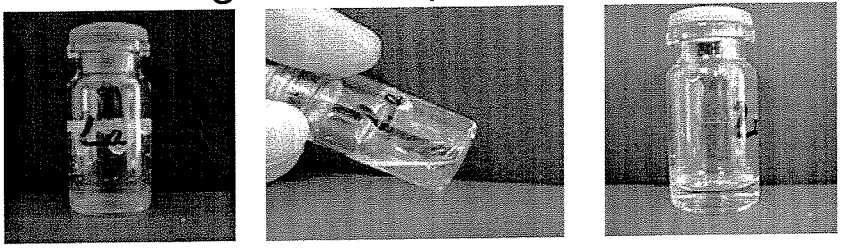
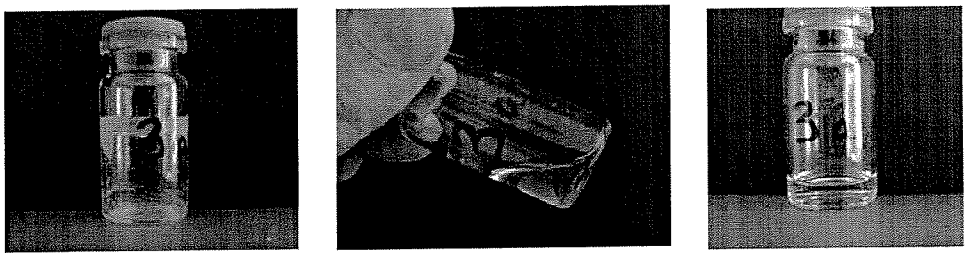


Figure to Experiment 2

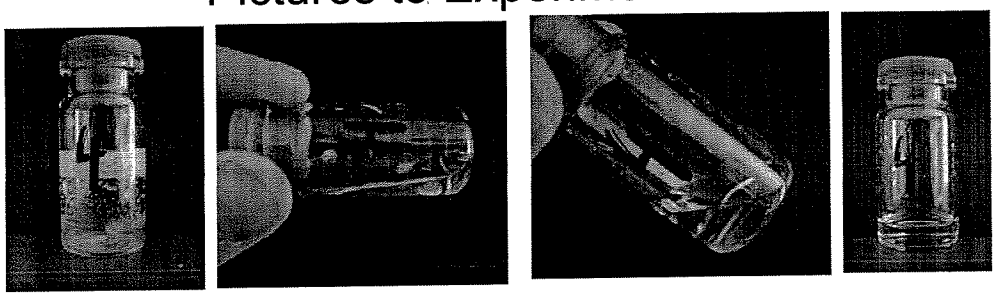


Pictures to Experiment 3a



Pictures to Experiment 3b

Pictures to Experiment 4



11. RELATED PROCEEDINGS APPENDIX

none